



Design, Synthesis and Biological evaluation of a bivalent μ opiate and adenosine A1 receptors antagonist

Smitha C. Mathew, Nandita Ghosh, Youlet By, Aurélie Berthault, Marie-Alice Virolleaud, Louis Carrega, Gaëlle Chouraqui, Laurent Commeiras, Jocelyne Condo, Mireille Attolini, et al.

► To cite this version:

Smitha C. Mathew, Nandita Ghosh, Youlet By, Aurélie Berthault, Marie-Alice Virolleaud, et al.. Design, Synthesis and Biological evaluation of a bivalent μ opiate and adenosine A1 receptors antagonist. *Bioorganic and Medicinal Chemistry Letters*, 2009, 19, pp.6736-6739. 10.1016/j.bmcl.2009.09.112 . hal-00679622

HAL Id: hal-00679622

<https://hal.science/hal-00679622>

Submitted on 16 Mar 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Design, Synthesis and Biological evaluation of a bivalent μ opiate and adenosine A₁ receptors antagonist

Smitha C. Mathew,^a Nandita Ghosh,^a Youlet By,^b Aurélie Berthault,^a Marie-Alice Virolleaud,^a Louis Carrega,^b Gaëlle Chouraqui,^a Laurent Commeiras,^a Jocelyne Condo,^b Mireille Attolini,^a Anouk Gaudel-Siri,^a Jean Ruf,^b Jean-Luc Parrain,^{a*} Jean Rodriguez,^{a*} Régis Guieu.^{b,c*}

^a Aix-Marseille Université, Institut des Sciences Moléculaires de Marseille, iSm2 – UMR CNRS 6263, Centre Saint Jérôme, Service 531, 13397 Marseille Cedex 20, France

^b Université de la Méditerranée, UMR MD2, Faculté de Médecine, boulevard Jean Moulin, 13005 Marseille, France

^c Assistance Publique des Hôpitaux de Marseille, boulevard Jean Moulin, 13005 Marseille, France

Abstract— We designed and synthesized a new hetero-bivalent ligand having antagonist properties on both A₁ adenosine and μ opiate receptors with a K_i of 0.8±0.05 and 0.7±0.03 μ M, respectively. This bipolar compound increases cAMP production both in cells over expressing the μ receptor and in those over expressing the A₁ adenosine receptor and reverses the antalgic effects of μ and A₁ adenosine receptors agonist in animals.

The cross talk between different G protein-coupled receptors (GPCRs) is the source of increasing research in the area of simultaneously targeting more than one GPCR.¹ Most of cross talk between different GPCRs concerns cAMP production *via* the modulation of adenylyl-cyclase activity. There is a great number of GPCRs that either stimulates or inhibits adenylyl-cyclase activity, depending on the nature of the G protein (G_s, G_i or other). Among GPCRs, there is evidence that A₁ARs and MORs are implicated in such a cross talk and their activation decrease cAMP level in the target cells.

In peripheral nervous system, there is a cross-tolerance and cross withdrawal between A₁ARs and MORs, indicating that these receptors are localized on the same primary afferent nociceptors and that A₁ARs and MORs cooperate as a multiple receptor complex form.² Our group has also demonstrated that the activation of MORs increased adenosine concentration in extra cellular spaces of the central nervous system,

suggesting that most effects of opioids are due to adenosine release.³

Based on the A₁ARs/MORs cross talk, the aim of this study was to design and to synthesize a potential hetero-bivalent A₁ARs/MORs ligand and to evaluate its biological effects. Hetero-bivalent ligand is a single chemical entity that is composed of two covalently linked pharmacophores with a dual mode of action, acting on two different receptor subtypes.^{1a,1f,4}

Modulating both A₁ARs and MORs could have therapeutic application in some diseases. As an example, the release of adenosine aggravated the hypotension during severe sepsis or septic shock, leading to subsequent tissue hypoperfusion and ischemia. Most of these effects are secondary to the activation of A₁ adenosine receptors, explaining the absence of response to pressive amines in these patients.⁵ Furthermore, naloxone, a μ receptor antagonist has been successfully used against the drop of blood pressure during septic shock⁶ or

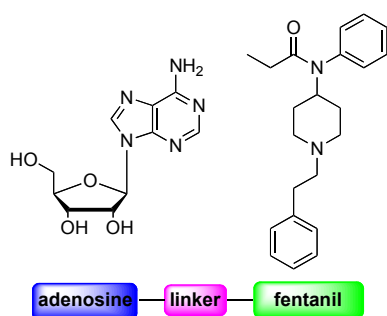
* Corresponding author. RG : Tel: 33 4 32 43 82 ; fax: 33 4 91 ; e-mail: guieu.regis@numericable.fr , JLP. Tel: 33 4 91 28 89 14; fax: 33 4 91 28 91 87; e-mail: jl.parrain@univ-cezanne.fr; JR Tel: 33 4 91 28 89 33; fax: 33 4 91 28 91 87; e-mail: jean.rodriguez@univ-cezanne.fr

hypovolemic shock,⁷ suggesting that the release of endogenous opioids participates into the severity of these syndromes. Thus blocking both A₁ ARs and MORs may be of a great interest in these pathologies. Modulating both these receptors may also be of interest in the area of drug-withdrawal therapy.⁸

We planned to associate Fentanyl [N-phenyl-N-(1-phenethyl-4-piperidiny)propanamide] to the adenosine molecule itself (Figure 1). Indeed, Fentanyl is one of the most powerful opioid analgesics, with a potency approximately eighty times that of morphine and forty times that of oxycodone.⁹ Herein, we report the synthesis of a bivalent compound (**10**), its μ opioid (MORs) and A₁ adenosine (A₁AR) receptors binding affinities and its biological properties.

Having in mind that the main approach to discovering AR agonists has been the modification of adenosine itself and that N⁶-cyclopentyladenosine (CPA) derivative displays high A₁AR selectivity,¹⁰ we first turned our attention to the synthesis of compound **10** presenting a cyclopentyl substitution at the adenosine N⁶-position and an amide bond linker at the 5'-position.

Figure 1. Concept of hetero-bivalent A₁ARs/MORs ligand



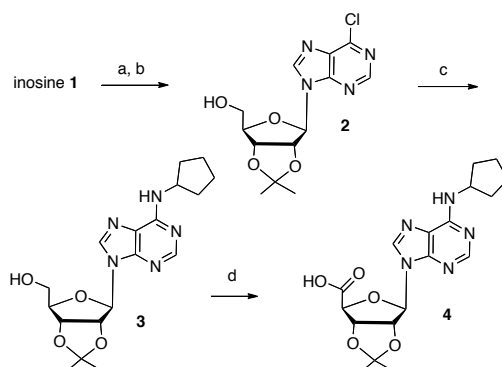
Our retrosynthetic approach for this potential hetero-bivalent ligand could involve an amidification step between the adenosine carboxylic acid **4** and the methyl amino fentanyl derivative **8** (Schemes 1 and 2).

Synthesis of acid **4** (Scheme 1) was now needed and started with the conversion of inosine **1** into the corresponding chloro acetal derivative **2**. Preparation of chloroinosine began with the acetylation of inosine with acetic anhydride in the presence of triethylamine and DMAP to afford triacetylinosine in quantitative yield. The conversion into the chloride with thionyl chloride was directly followed by a deacetylation using a

solution of ammonia in methanol to give the expected chloroinosine (90% yield over 3 steps). Selective protection of the two secondary alcohol functions was achieved with dimethoxypropane in presence of *p*-TSA to give **2** in 70%.¹¹ After being subjected to cyclopentylamine in the presence of calcium carbonate, compound **2** led to the corresponding secondary amine **3** in 98% yield. Thereafter the primary alcohol underwent an oxidation to the acid **4** under TEMPO-iodobenzene diacetate conditions¹² in 65% yield. Attention was next turned to the fentanyl part of target molecule **8** (Scheme 2). Reductive amination of 4-aminoacetophenone with commercially available 1-phenethylpiperidine-4-one (**5**) afforded amine **6**.

This secondary amine was next subjected to propionylation and led to the formation of the desired compound **7** in 95% yield.

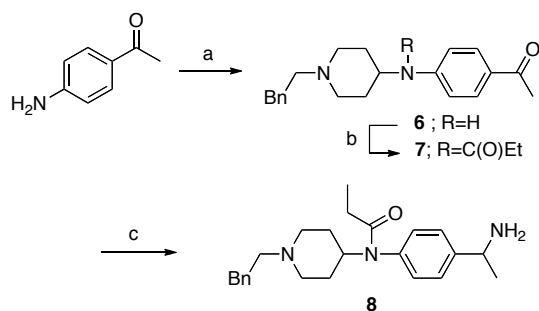
Scheme 1. Synthesis of adenosyl carboxylic acid **4**



Reagents and conditions: (a) (1) Ac₂O, DMAP, Et₃N, ACN, rt, quant.; (2) SOCl₂, DMF, DCM, 0 °C then reflux, 90%; (3) NH₃/MeOH, 0 °C then 20 °C, quant. (b) dimethoxypropane, *p*-TSA, acetone, rt, 70%; (c) cyclopentylamine, DIEA, EtOH, 80°C, 98%; (d) BAIB, TEMPO, ACN/H₂O, rt, 65%.

The latter in the presence of ammonium acetate and sodium cyanoborohydride furnished subunit **8** in 55% yield.¹¹

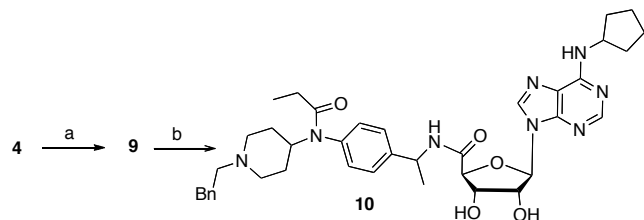
Scheme 2. Synthesis of methylaminofentanyl **8**



Reagents and conditions: (a) **5**, NaBH(OAc)₃, AcOH, DCE, rt, 77%; (b) propionyl chloride, Et₃N, DCM, reflux, 95%; (c) NH₄OAc, NaBH₃CN, MeOH, 50 °C, 55%.

The synthesis of target **10** (Scheme 3) was completed in two steps by using a BOP-assisted coupling between acid **4** and amine **8**¹³ then a deprotection of the acetal moiety using aqueous trifluoroacetic acid affording the target molecule **10** with good overall yield. By using this approach this potential hetero-bivalent ligand was efficiently synthesized on milligram scale (93% HPLC purity grade) in 8 steps.

Scheme 3. Synthesis of adenosyl fentanyl **10**



Reagents and conditions: (a) **7**, BOP, Et₃N, THF, rt, 77%; (b) 80% TFA, rt, 32%.

This new hetero-bivalent ligand **10** was then submitted to biological evaluation.¹⁴ Adenosyl fentanyl **10** was first tested using nociceptive tests in animals. Adenosyl fentanyl **10** alone had no effect on latencies.

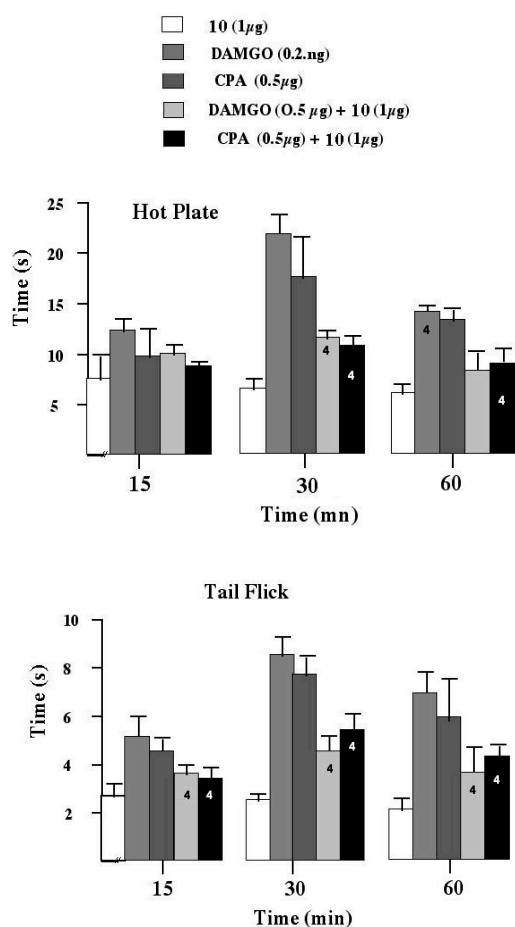


Figure 2. Results of nociceptive tests in animals

However it is worth to note that DAMGO, a well known synthetic opioid peptide with μ agonist properties, co-injected with **10** showed shorter latencies than DAMGO alone both in hot plate and tail flick tests. Adenosyl fentanyl **10** also reversed significantly the increase of latencies induced by *N*₆ cyclopentyl adenosine (CPA), an A1 AR agonist (Figure 2). Comparable results were obtained using intraperitoneal administration of drugs (data not shown). We also evaluated the acute toxicity of **10** in animals: LD 50 value was >50 µg/mouse (when icv administered) and > 500 mg/kg (when ip administered). We tested the properties of **10** to modulate cAMP production in cell culture. **10** Activated cAMP production in a dose dependent manner both in CHO K1 cells that over expressed MORs and in CHO Chem 3 cells that over expressed A1ARs, with a maximal stimulation of 29±7% and 17±3%, respectively.

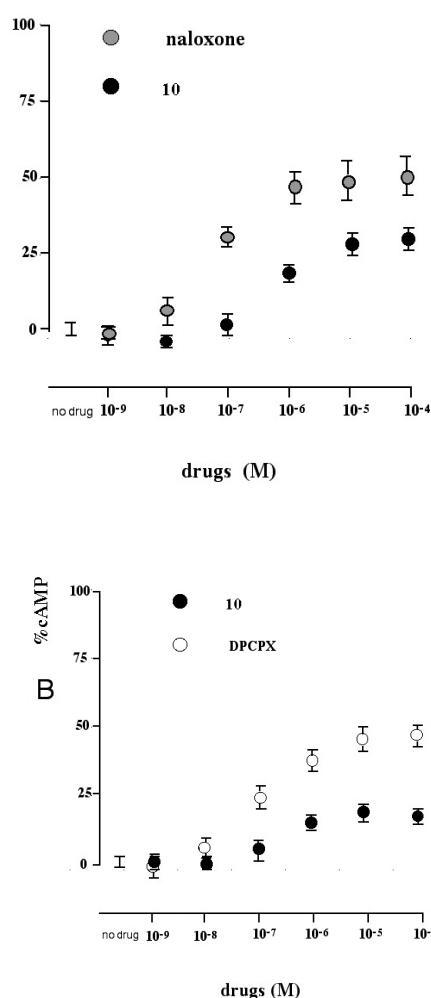


Figure 3. Comparative effects of **10**, naloxone and DPCPX on cAMP production

Comparatively, naloxone, a MORs antagonist, increased cAMP production with a maximal stimulation of $50 \pm 16\%$ while 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX), an A₁ AR antagonist, increased cAMP production with a maximal stimulation of $45 \pm 8\%$ (Figure 3).

We also evaluated the affinities (K_i) of drugs tested, using binding assay on cell membranes (Table). **10** had substantial lower affinities for the A₁ARs as compared with A₁ARs antagonist DPCPX and/or lower affinities for the MORs as compared with the MORs antagonist naloxone or with the MORs agonist DAMGO. However, its affinity was sufficient to produce biological effects at reasonable concentrations.

Table 1: K_i values for drugs at the A₁ AR and the MORs

K _i A ₁ AR	K _i A _{2A}	K _i MORs
----------------------------------	--------------------------------	---------------------

	(nM)	AR (nM)	(nM)
DPCPX	12.9±3		
Naloxone			1.5±0.2
DAMGO			3.8±0.7
10	800±57	>40.000	728±37

DPCPX: 8-Cyclopentyl-1,3-dipropylxanthine (A₁ AR antagonist) Naloxone: MORs antagonist; DAMGO: ([D-Ala², N-Me Phe⁴, Gly-ol]enkephalin)(MORs agonist)

We have synthesized a hetero-bivalent ligand **10** that have antagonist properties on both A₁AR and MORs. Its affinity remained low for the two receptors but sufficient to have biological effects at reasonable concentration. **10** Reversed significantly the antalgic effects of DAMGO or CPA and activated cAMP production in both cell lines tested. To the best of our knowledge, adenosyl fentanyl **10** constitutes the first drug that blocks both MORs and A₁ARs. Multivalent-ligands are also called hybrid molecules, and are defined as chemical entities having different biological effects.^{4c} There is evidence that adenosine *via* A₁ARs, is implicated in the modulation of opiates action in the central nervous system.²⁴ Morphine²⁵ and adenosine²⁶ inhibit Ca²⁺ dependent NT release; this inhibition is blocked by theophylline an adenosine receptors antagonist.²⁰ A₁ARs and MORs are both implicated in septic or hypovolemic shock,^{5,6,7} withdrawal,^{8,21} mood regulation²² and pain.^{24,30} Thus to have drugs which modulate both A₁AR and MORs at one's disposal, could be interesting in these area. After these encouraging preliminary results we have embarked in more systematic SAR study in design more active and selective hybrid targets. These results will be reported in due course.

Acknowledgments

The authors thank Agence Nationale pour la Recherche (ANR-05-BLAN-0175-01), CNRS, Université Paul Cézanne, Université de la Méditerranée, Faculté de Médecine, Assistance Publique des Hôpitaux de Marseille, Fondation Mérieux and Conseil Régional Provence Alpes Côte d'Azur for fundings; Johnson and Johnson laboratories for CHO K1 cells supply; Conseil Général des Bouches du Rhône are also gratefully acknowledge; Drs T. Meert and van Hijfte, and Miss Dechabanne for helpful suggestions.

References

1. (a) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Yim, C. B.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. *J. Med. Chem.* 1986, **29**, 1855. (b) Jacobson, K. A.; Xie, R.; Young, L.; Chang, L.; Liang, B. T. *J. Biol. Chem.* 2000, **275**, 30272. (c) Daniels, D. J.; Kulkarni, A.; Xie, Z.; Bhushan, R. G.; Portoghese, P. S. *J. Med. Chem.* 2005, **48**, 1713. (d) Savini, L.; Campiani, G.; Gaeta, A.; Pellerano, C.; Fattorusso, C.; Chiasserini, L.; Fedorko, J. M.; Saxena, A. *Bioorg. Med. Chem. Lett.* 2001, **11**, 1779. (e) Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. *J. Med. Chem.* 2006, **49**, 256. (f) Karellas, P.; McNaughton, M.; Baker, S. P.; Scammells, P. *J. J. Med. Chem.* 2008, **51**, 6128.
2. Aley, K. O.; Levine, J. D. *J. Neurosci.* 1997, **17**, 735.
3. Halimi, G.; Devaux, C.; Clot-Faybesse, O.; Sampol, J.; Legof, L.; Rochat, H.; Guieu, R. *Eur. J. Pharmacol.* 2000, **398**, 217.
4. (a) Perez, M.; Jorand-Lebrun, C.; Pauwels, P. J.; Pallard, I.; Halazy, S. *Bioorg. Med. Chem. Lett.* 1998, **8**, 1407. (b) Bhushan, R. G.; Sharma, S. K.; Xie, Z.; Daniels, D. J.; Portoghese, P. S. *J. Med. Chem.* 2004, **47**, 2969. (c) Meunier, B. *Acc. Chem. Res.* 2008, **41**, 69.
5. Martin, C.; Leone, M.; Viviani, X.; Ayem, M. L.; Guieu, R. *Crit Care Med.* 2000, **28**, 3198.
6. Peters, W. P.; Johnson, M. W.; Friedman, P. A.; Mitch, W. E. *Lancet* 1981, **1**, 529.
7. Faden, A. I.; Holaday, J. W. *Science* 1979, **205**, 317.
8. Khorasani, M. Z.; Hajizadeh, S.; Fathollahi, Y.; Semnani, S. *Brain Res.* 2006, **1084**, 61.
9. Fentanyl: Incapacitating Agent. National Institute for Occupational Safety and Health. Emergency Response Database. August 22, 2008. Retrieved December 31, 2008.
10. Ju, Y.; Kumar, D.; Varma, R. S. *J. Org. Chem.* 2006, **71**, 6697.
11. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* 1996, **61**, 3849.
12. Boger, D. L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc.* 1994, **116**, 8544.
13. Borch, R. F.; Bernstein, M. D.; Dupont Durst, H. *J. Am. Chem. Soc.* 1971, **93**, 2897.
14. Jacobson, K.; Gao, Z.-G. *Nature Reviews* 2006, **5**, 247.
15. Koufaki, M.; Theodorou, E.; Galaris, D.; Nousis, L.; Katsanou, E. S.; Alexis, M. N. *J. Med. Chem.* 2006, **49**, 300.
16. Kogen, H.; Toda, N.; Tago, K.; Marumoto, S.; Takami, K.; Ori, M.; Yamada, N.; Koyama, K.; Naruto, S.; Abe, K.; Yamazaki, R.; Hara, T.; Aoyagi, A.; Abe, Y.; Kaneko, T. *Org. Lett.* 2002, **4**, 3359.
17. Salem, A.; Hope, W. *Pharmacol. Biochem. Behav.* 1997, **57**, 671.
18. Michaelson, D. M.; McDowall, G.; Sarne, Y. *J. Neurochem.* 1984, **43**, 614.
19. Silinsky, E. M. *J. Physiol.* 2004, **558**, 389-401.
20. Cahill, C. M.; White, T. D.; Sawynok, J. *J. Pharmacol. Exp. Ther.* 1995, **275**, 84.
21. Hack, S. P.; Christie, M. J. *Crit. Rev. Neurobiol.* 2003, **15**, 235.
22. Kaster, M. P.; Budni, J.; Santos, A. R.; Rodrigues, A. L. *Eur. J. Pharmacol.* 2007, **576**, 91.
23. Guieu, R.; Peragut, J. C.; Roussel, P.; Hassani, H.; Sampieri, F.; Bechis, G.; Gola, R.; Rochat, H. *Pain* 1996, **68**, 271.
24. Pham, T.; Carrega, L.; Sauze, N.; Fund-Saunier, O.; Devaux, C.; Peragut, J. C.; Saadjian, A.; Guieu, R. *Anesthesiology* 2003, **98**, 459-464.

Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.